

Functional properties of Australian blue lupin (*Lupinus angustifolius*) protein and biological activities of protein hydrolysates

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Abstract

Lupin is an undervalued legume despite its high protein content with known health benefits. In this research, Australian blue lupin protein was isolated and hydrolysed enzymatically to produce bioactive peptides with a view to assess their potential for nutraceutical and therapeutic applications. Pepsin, pancreatin and flavourzyme were used to enzymatically hydrolyse blue lupin protein, and the hydrolysates were subjected to molecular weight cut-off (MWCO) fractionation. Measurement of biological activities led to the identification of angiotensin converting enzyme (ACE) inhibitory fractions in the molecular weight range of 2–3 and 3–5 kDa. For the most active fractions in this range, the ACE inhibitory activities were very significant with IC_{50} values from 450 to 600 $\mu\text{g/ml}$. Blue lupin protein-derived MWCO fractions were significantly active against Gram-positive bacteria and only a little inhibition was observed against Gram-negative bacteria. Pancreatin hydrolysed fractions showed the best antimicrobial activities with several fractions exhibiting $\geq 85\%$ inhibition against *Bacillus cereus* and *Staphylococcus aureus*. These properties reveal the potential of lupin protein hydrolysates for developing antihypertensive and host defence agents. In order to demonstrate the potential of isolated blue lupin protein in food industry, functional properties including water and oil absorption capacity, gelling properties, solubility and emulsifying properties were evaluated and found to be extremely suitable for developing functional foods with enhanced health benefits.

KEYWORDS

ACE inhibitory activity, antimicrobial properties, Australian blue lupin protein, functional properties

1 | INTRODUCTION

Consumption of food protein results in beneficial effects on human health. These proteins are derived from animal and plant sources, for example, milk, eggs, cheese, meat, fish, soy, rice, wheat and legumes (Kamran & Reddy, 2018; Kamran, Salampessy, & Reddy, 2016; Korhonen & Pihlanto, 2006; Pritchard, Phillips, & Kailasapathy, 2010).

The beneficial effects of these food proteins are enhanced either by gastrointestinal enzymatic hydrolysis or by microbial fermentation to generate peptides with improved biological activities. Fermented soybean products, such as soy sauce, natto and tempeh, have been widely studied for their antihypertensive activities (Gibbs, Zougman, Masse, & Mulligan, 2004; Kamran & Reddy, 2018; Kuba, Tana, Tawata, & Yasuda, 2005; Kuba, Tanaka, Tawata, Takeda, &

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Yasuda, 2003; Rho, Lee, Chung, Kim, & Lee, 2009; Sanjukta & Rai, 2016). Literature demonstrates that enzymatically derived peptides from food proteins display antihypertensive, osteoprotective, antioxidant, antimicrobial, anti-inflammatory, anticancer, growth promoting and many other positive effects on human health when they are absorbed from intestinal tract or via receptors (Boschin, Scigliuolo, Resta, & Arnoldi, 2014a; Möller, Scholz-Ahrens, Roos, & Schrezenmeir, 2008; Paiva, Lima, Neto, & Baptista, 2016).

Lupin seeds have gained popularity as health food items and research on their application as ingredients in food production continues to emerge. Special focus has been on lupin seed protein that exhibits beneficial functional and nutraceutical properties when incorporated into food products such as bread, pasta, biscuits, soups and salad dressing (Johnson, McQuillan, Sin, & Ball, 2003; Lee et al., 2009; Torres, Frias, Granito, Guerra, & Vidal-Valverde, 2007). For instance, incorporation of lupin flour into wheat flour increases the protein and fibre content and improves the amino acid profile of food products (Jayasena, Leung, & Nasar-Abbas, 2010; Jayasena & Nasar-Abbas, 2011; Villarino, Jayasena, Coorey, Chakrabarti-Bell, & Johnson, 2015). Lupin protein has been shown to have good solubility, foaming, gelling and emulsifying properties that are comparable with other legumes and soybean and hence has been used to develop various types of food products (Bader, Oviedo, Pickardt, & Eisner, 2011).

The angiotensin converting enzyme (ACE) inhibitors reduce the activity of ACE and produce a vasorelaxing effect on blood vessels (Barbana & Boye, 2011; Kamran, Salampessy, & Reddy, 2016; Kuba, Tana, Tawata, & Yasuda, 2005; Nasri et al., 2013; Paiva, Lima, Neto, & Baptista, 2016; Rho, Lee, Chung, Kim, & Lee, 2009; Shahidi & Zhong, 2008). Therefore, inhibition of ACE is very important in regulating blood pressure in hypertensive subjects (Boschin, Scigliuolo, Resta, & Arnoldi, 2014a; Chin et al., 2019; Hong et al., 2008; Lavoie & Sigmund, 2003; Luna-Vital, Mojica, González de Mejía, Mendoza, & Loarca-Piña, 2015; Mäkinen, Streng, Larsen, Laine, & Pihlanto, 2016; Shahidi & Zhong, 2008). During the past decade, several ACE inhibitory peptides (ACEIPs)/antihypertensive peptides were derived from various food proteins with significant activity in reducing blood pressure and treating cardiovascular diseases (Balti et al., 2015; Boschin, Scigliuolo, Resta, & Arnoldi, 2014a; Nongonierma & FitzGerald, 2016; Shahidi & Zhong, 2008). Therefore, the research on lupin protein hydrolysates aimed to develop natural antihypertensive agents with low side effects is of enormous importance (Boschin, Scigliuolo, Resta, & Arnoldi, 2014a; Paiva, Lima, Neto, & Baptista, 2016). It has been shown that the pepsin hydrolysates of protein extracted from the three most commonly consumed lupin species (*Lupinus albus*, *Lupinus angustifolius* and *Lupinus luteus*) have exhibited ACE inhibitory activities with low IC₅₀ (mean value of three species is $186 \pm 10 \mu\text{g/ml}$) indicating that lupin proteins/peptides may be a valuable source of ACE inhibitory peptides (Arnoldi, Boschin, Zanoni, & Lammi, 2015; Boschin, Scigliuolo, Resta, & Arnoldi, 2014b; Kamran, 2017). Studies on de-oiled *L. albus* protein isolate demonstrated that pepsin plus pancreatin hydrolysate showed potent (58.7%) ACE inhibition (Boschin, Scigliuolo, Resta, & Arnoldi, 2014b; Yoshie-Stark, Bez, Wada, & Waesche, 2004).

Antimicrobial peptides (AMPs) have been the focus of research around the world due to their potential application to combat the emergence of antibiotic resistant pathogenic microorganisms. The AMPs can exist naturally and are derived from food protein substrates and known as nature's antibiotics (Hancock & Chapple, 1999; Tang et al., 2015). Research on AMPs from blue lupin protein is scarce in literature. Yeo, Lee, Cha, and Hahm (2011) have identified a thermally stable AMP, AMP IC-1, from Korean traditional fermented soybean paste. Its activity was comparable with the previously reported peptide (BSAP-254) against *Bacillus cereus* (Sanjukta & Rai, 2016; Yeo, Lee, Cha, & Hahm, 2011). The purified AMP IC-1 is a 33 amino acid sequence with 13 different residues (namely, Cys, Asn or Asp, Gln or Glu, Ser, Ala, Pro, Gly, Arg, Thr, Val, Ile, Leu and Lys). The amino acids in both of these AMPs are very similar except for some minor differences (Sanjukta & Rai, 2016; Yeo, Lee, Cha, & Hahm, 2011). A novel inhibitor mungoin, derived from mung bean (*Phaseolus mungo*) seeds, has displayed significant antifungal and antibacterial activities (S. Wang et al., 2006). It exerted a potent inhibitory action toward a variety of fungal species including *Phylospora piricola*, *Mycosphaerella arachidicola*, *Botrytis cinerea*, *Pythium aphanidermatum*, *Sclerotium rolfsii* and *Fusarium oxysporum*, as well as an antibacterial action against *Staphylococcus aureus* (S. Wang et al., 2006; S. Y. Wang, Wu, Ng, Ye, & Rao, 2004). In addition, this novel plant protease inhibitor displayed anti-proliferative activity towards tumour cells (S. Wang et al., 2006).

In this paper, the term lupin protein refers to the protein isolated from blue lupin seeds. To the best of our knowledge, there is no report on the discovery of AMPs from lupin protein hydrolysates. There is no literature involving any attempts to study antimicrobial and antifungal potentials of lupin protein-derived peptides. Also, very limited information on the production of bioactive peptides with ACE inhibitory activity is available in the literature. Therefore, this research has two major aims: (i) isolation of blue lupin protein and evaluation of their functional properties and (ii) enzymatic hydrolysis of blue lupin protein and the investigation of ACE inhibitory and antimicrobial properties of these hydrolysates.

2 | MATERIAL AND METHODS

2.1 | Chemicals and consumables

Angiotensin I-converting enzyme from rabbit lung and the ACE synthetic substrate hippuryl-L-histidyl-L-leucine (HHL), HEPES (4-(2-hydroxyethyl) piperazine-1-ethanesulfonic acid sodium salt), captopril, sodium chloride (NaCl), sodium hydroxide (NaOH), hydrochloric acid (HCl), phosphate buffer, sodium dodecyl sulphate, 2-propanol, ethyl acetate, Bio-Rad protein assay dye, and bovine serum albumin for Bradford assay. All chemicals were of reagent grade and obtained from Sigma-Aldrich Chemical Company (Castle Hill, New South Wales, Australia).

Microbial strains used in this study were *Escherichia coli* 185 (ATCC 8739), *S. aureus* 184 (ATCC 6538), *B. cereus* 106 (ATCC

11778) and *Candida albicans* X9 from the culture collection of Western Sydney University, Hawkesbury Campus, Richmond, NSW, Australia. The BHI broth was prepared by dissolving 18.5 g BHI media powder (Difco, Sparks, NJ, USA) in distilled water to make 500 ml media solution. The media was sterilized at 121°C for 15 min and stored at 4°C until used. All bacterial strains were cultured from frozen stock by incubation overnight at 37°C in BHI broth. After overnight incubation, the culture was diluted with the broth and the turbidity was compared with equal that of 0.5 M McFarland standards that correspond to an optical density of about 0.1 at 595 nm.

The *C. albicans* culture was prepared by growing the stock fungal species on MRS (De Man, Rogosa, Sharpe) broth at 37°C overnight. The culture was diluted in the same broth to attain a final concentration of 10^7 CFU/mL that corresponded to an optical density reading of about 1.0 at 450 nm.

2.2 | Extraction of lupin protein

Australian sweet lupin (*L. angustifolius*) flour was obtained from Curtin University, Western Australia, and was prepared from de-hulled seeds. Lupin protein was isolated by alkaline water extraction and iso-electric precipitation by the method of Sironi, Sessa, and Duranti (2005) with some modifications. After defatting with 2-propanol (1:4 w/v), lupin flour was suspended in distilled water (1:10 w/v) and the pH of the suspension was adjusted to 9.0 using 1 M NaOH. The suspension was stirred for 1 h at room temperature and centrifuged at 10,000g for 30 min. The extraction steps were repeated twice for maximum yield. The supernatants were collected and acidified to pH 4.5, using 1 M HCl. The precipitate was recovered, neutralized and freeze dried for further studies (Sironi et al., 2005).

2.3 | Determination of functional properties of lupin protein

2.3.1 | Water and oil absorption capacities

Isolated lupin protein (0.5 g) was shaken vigorously for 1 min with 5 ml of water and corn oil each in two separate tubes and centrifuge for 25 min at 16,000g after standing the tubes for 30 min at room temperature. After centrifuging and filtration, the volume of liquid was measured and expressed as millilitres of water/oil absorbed per gram of protein (Rodriguez-Ambriz, Martinez-Ayala, Millan, & Davila-Ortiz, 2005).

2.3.2 | Protein solubility

The solubility of lupin protein isolate was measured by the method of King, Aguirre, and De Pablo (1985) with some modifications. Protein suspensions (0.5% w/v) were prepared at different pH values ranging from 2 to 10 by using 1 M HCl and 1 M NaOH and stirred for 60 min.

Centrifuged the mixtures at 16,000g for 15 min to remove solid content and observed the percentage protein by using Bradford assay at 595 nm. Solubility was calculated by the ratio of protein dissolved in supernatant to total protein in the initial sample. To study the effect of ionic strength on protein solubility, the above process was repeated by preparing suspensions with 1 M NaCl.

2.3.3 | Foaming property

Foaming properties were analysed by the method of Rodriguez-Ambriz, Martinez-Ayala, Millan, and Davila-Ortiz (2005) with minor modifications. Protein solutions, 50 ml (1% w/v) of pH values 2, 4, 6, 8 and 10 were stirred by electric blender for 5 min. The volume of foam was measured by immediately pouring the solution into 250-ml graduated glass cylinder, and the volume was recorded. The percent increase in foam volume was measured as foam capacity, and the volume of remaining foam after standing 60 min at room temperature was recorded to calculate foam stability:

$$\text{Foam stability (\%)} = [\text{Foam volume after time } t \text{ (min)} / \text{Initial foam volume}] \times 100.$$

2.3.4 | Emulsifying property

The emulsifying property was determined using the method of Pearce and Kinsella (1978) by preparing 4.5 ml of 0.5% (w/v) protein solution in 0.01 M phosphate buffer (pH 7). To this solution 1.5 ml of corn oil was added and homogenized for 1 min at 20,000 rpm using a homogenizer. Dilutions were made after different time intervals by taking 250 μ l of emulsion and adding in 50 ml of 0.1% sodium dodecyl sulphate solution. The absorbance of the diluted emulsions was measured at 500 nm by multiwell plate reader, and emulsifying activity and stability was calculated (Can Karaca, Low, & Nickerson, 2011; Pearce & Kinsella, 1978).

2.3.5 | Gelling property

The gelling property of lupin protein isolate was determined by the method of Rodriguez-Ambriz, Martinez-Ayala, Millan, and Davila-Ortiz (2005). The protein suspensions of 4%, 6%, 8%, 10% and 12% were prepared with MQW (5 ml each) and the test tubes were heated in boiling water bath for 1 h. The test tubes were then rapidly cooled under running tap water and then for up to 2 h at 4°C. The least gelation concentration was determined from the sample that did not fall out when test tube inverted.

2.4 | Enzymatic hydrolysis of lupin protein

Lupin protein isolates were digested with pepsin and pancreatin (enzyme/substrate ratio = 1:200) at 37°C and at pH 2 and

7, respectively. Hydrolysed samples were collected at hourly intervals for 4 h (Yoshie-Stark, Bez, Wada, & Waesche, 2004). For the digestion with flavourzyme the conditions were enzyme/substrate ratio of 1:10, pH 8 and 50°C. Samples were collected at hourly intervals for 4 h (Barbana & Boye, 2011). The hydrolysates were subjected to 10, 5, 3 and 2 kDa VivaSpin molecular weight cut-off (MWCO) membranes to separate peptide fractions of different molecular weights. A total of 48 MWCO fractions were obtained and were further analysed for their bioactivities.

2.5 | Determination of ACE inhibitory activity

The ACE inhibitory activity was measured in vitro by following the method of Meira et al. (2012) and Nakamura et al. (1995) (Meira et al., 2012; Nakamura et al., 1995) with some modifications. Each peptide fraction (2 mg/ml) of 40 µl were added to 100 µl of buffered substrate solution, that is, 5 mM hippuryl-histidyl-leucine (Sigma-Aldrich) in 50 mM HEPES buffer containing 300 mM NaCl, pH 8.3. The reaction was started by addition of 20 µl of ACE (0.1 U/ml, from rabbit lung, Sigma-Aldrich), incubated at 37°C for 30 min, and then finished with 150 µl of 1 M HCl. Then, the hippuric acid released was extracted with 1 ml of ethyl acetate by vortex mixing for 20 s and centrifugation for 3 min at 10,000 rpm. The organic phase was aliquoted and transferred to a glass tube to be heat evaporated. The residue was dissolved with 800 µl of distilled water and measured spectrophotometrically at 228 nm. The activity of each sample was tested in triplicate. The assay mixture without protein hydrolysate was used as a blank. The ACE inhibitory activity was expressed as a percentage using the formula:

$$\% \text{Inhibitory Activity} = (A_{\text{blank}} - A_{\text{sample}} / A_{\text{blank}}) \times 100.$$

The activity of lupin hydrolysates was expressed as the concentration of protein needed to inhibit 50% of ACE activity (IC₅₀). Captopril (1 mM, Sigma-Aldrich) was used as a positive ACE inhibitor control in this assay.

2.6 | Determination of antimicrobial activity

Antimicrobial assays were carried out using *E. coli* 185, *S. aureus* 184, *B. cereus* 106 and *C. albicans* X9 on a multiwell plate using BHI broth, by the method of Andrews (2001). BHI broth (90 µl) was added to each well of multiwell plate containing 50 µl of peptide extract (1 mg/ml). Each bacterial culture (10 µl) was added in separate assays. Positive control was obtained by using tetracycline (64.0 mg/L), and negative control was used as sterile water with the bacterial suspension. All preparations were carried out in triplicate on a 96-well plate and the absorbance at 595 nm was read after 24-h incubation. Percentage of inhibition was determined by

$$\% \text{Inhibition} = \frac{A_{\text{Sample}} - A_{\text{Negative Control}}}{A_{\text{Positive Control}} - A_{\text{Negative Control}}} \times 100.$$

Antifungal activity was performed against *C. albicans* by employing standard method.

2.7 | The MIC assay

The minimal inhibitory concentration (MIC) of selected fractions was observed and quantified spectrophotometrically on a microplate reader (Bio-Rad Benchmark Plus). The peptide samples were diluted with different concentrations as their final concentrations were 660, 330, 165, 83, 41 and 21 µg/ml; 50 µl of each fraction was added in 90 µl of BHI broth in a multiwell plate. Bacterial culture (10 µl) was added in the mixture and its optical density was measured after 24-h incubation at 37°C. Tetracycline was used as positive control and sterile water was used as negative control. The MIC was calculated as the lowest concentration of the peptide to completely inhibit the growth of the microorganisms (Salampessy, Phillips, Seneweera, & Kailasapathy, 2010).

2.8 | Statistical analysis

Statistical analysis of the results was carried out using SPSS (Version 22, IBM SPSS, Chicago, IL, USA) and Microsoft Excel. The triplicate data were expressed as mean ± standard deviation values. The group mean was compared using a one-way analysis of variance and Duncan's multiple range tests. All the results were found to be statistically significant as *p* values were <0.05.

3 | RESULTS AND DISCUSSION

3.1 | Functional properties of lupin protein

Protein is an important ingredient in food product development. Its composition has significant influence on consumer health and acceptability. Functional properties of food protein such as solubility, water holding, oil binding, foaming, emulsifying and gelation capacities have significant impact on the food product quality. These properties of blue lupin protein are presented and discussed in this section.

3.1.1 | Water and oil absorption capacities

Isolated blue lupin protein was estimated for water and oil absorption capacities. It showed 1.16 ml/g water absorption and 3.57 ml/g oil absorption capacities (Table 1). Sathe, Deshpande, and Salunkhe (1982) have reported 1.37 ml/g water absorption and 3.25 ml/g oil absorption capacities for lupin protein which are comparable with the results reported in this paper. Rodriguez-Ambriz, Martinez-Ayala, Millan, and Davila-Ortiz (2005) isolated *Lupinus campestris* protein by isoelectric

TABLE 1 Functional properties of lupin protein ($n = 3$; $p \leq 0.05$)*

Functional properties	pH = 2	pH = 4	pH = 6	pH = 8	pH = 10
Water absorption capacity (ml/g)	1.16 ± 0.49				
Oil absorption capacity (ml/g)	3.57 ± 0.41				
Emulsifying activity (m ² /g)	41.78 ± 0.82				
Emulsifying stability (min)	15.34 ± 0.87				
Least gelling capacity (w/v %)	8% protein solution				
Protein solubility with NaCl (%)	19.76 ± 0.15	7.25 ± 0.08	67.95 ± 0.31	75.22 ± 0.42	95.02 ± 0.16
Protein solubility without NaCl (%)	19.67 ± 0.06	17.54 ± 0.17	9.13 ± 0.17	23.54 ± 0.40	25.92 ± 0.14
Foaming capacity (%)	124 ± 1.35	120 ± 2.31	116 ± 2.85	118 ± 1.92	128 ± 3.01
Foaming stability (%)	91.60 ± 1.55	88.88 ± 1.68	88.88 ± 2.10	87.50 ± 1.21	76.92 ± 1.12

Note: *All values are mean of triplicate determination ± standard deviation.

method and demonstrated that both water and oil absorption capacities of lupin protein isolate was 1.7 mL/g whereas for soybean protein, these properties were 2.2 and 1.5 mL/g of protein, respectively. These properties of lupin are comparable with that of soybean protein.

Water absorption capacity depends on polarity, size and shape of amino acid residues which determine the extent of interaction of protein molecules with polar water molecules, whereas the fat absorption capacity depends on nonpolar amino acid side chains within the protein molecules that interact with hydrocarbon chains of fat molecules. The high water and oil absorption capacities are beneficial for food product manufacturing because they improve the shelf life and organoleptic properties of food products (Rodriguez-Ambriz, Martinez-Ayala, Millan, & Davila-Ortiz, 2005; Tizazu & Emire, 2010).

3.1.2 | Protein solubility

Blue lupin protein solubility is shown in Table 1. The results showed that the solubility of isolated protein is highest at pH 10 (95%) and it is lowest at pH 4 (7.25%) in 1 M NaCl (aq) solution. Literature suggests that the protein solubility reaches its maximum in the presence of moderate concentration of ionic salt solution (~1 M) and decreases when the strength of salt solution is either lower or higher than 1 M (El-Adawy, Rahma, El-Bedawey, & Gafar, 2001; Yi-Shen, Shuai, & FitzGerald, 2018). The water suspensions without salt showed higher solubility in highly acidic and alkaline media whereas lowest solubility is observed at pH range 5.5–6. At pH 10, the solubility of protein is 25.9%; that is about 70% lower than that in ionic salt solution. These results are in agreement with those reported in the literature for protein isolated from *L. campestris* (King, Aguirre, & De Pablo, 1985; Rodriguez-Ambriz, Martinez-Ayala, Millan, & Davila-Ortiz, 2005; Sathe, Deshpande, & Salunkhe, 1982). Solubilities of isoelectrically precipitated proteins from other legumes, pea, chickpea and lentil were reported in the literature (Boye et al., 2010). The highest solubilities were observed in the pH ranges of 1 to 3 and 7 to 10, whereas the solubilities were very low in the pH range of 4 to 6. These observations are similar to the results presented in this paper for blue lupin protein (Pei Gee, Casey, & Johnson, 2003).

3.1.3 | Foaming property

Foaming properties of 1% (w/v) protein solution at different pH values are shown in Table 1. The results showed that the foaming capacity of blue lupin protein isolate is higher (124% and 128%) at acidic and alkaline regions (pH 2 and 10, respectively) and lower (116%) at the isoelectric region (pH 6). Six Mexican varieties of *L. angustifolius* were studied in the literature that exhibited lower foaming capacities in the range of 116.3 to 116.8% at neutral pH (Lara-Rivera et al., 2017). These results are consistent with the results presented in this paper for blue lupin protein.

The foam stability is highest at pH 2 (91.6%) after standing for 60 min, whereas at pH 10, the foam shows lowest stability (76.9%). Similar results were observed by Rodriguez-Ambriz, Martinez-Ayala, Millan, and Davila-Ortiz (2005); at pH 2, the foam stability was 85% for *L. campestris* protein isolate and 95% for soybean protein isolate. Lowest stability was reported at pH 8 and 10, that is, 70% for *L. campestris* and 25% for soybean protein isolate. The acidic pH provides a thick molecular layer in the air–water interphase, which retains texture, elasticity and stability of foam. Similar behaviour has been reported for lupin protein isolate to that of soybean, sunflower and other legume protein isolates (Rodriguez-Ambriz, Martinez-Ayala, Millan, & Davila-Ortiz, 2005; Sathe, Deshpande, & Salunkhe, 1982).

Foam formation depends on the ability of protein to reduce surface tension between two phases (air–water) and retain the film formed around the air bubble. Foaming property is important to make cake, ice cream, mousses, whipped cream, and so on (Chao, Jung, & Aluko, 2018; Smith, 2010).

3.1.4 | Emulsifying property

The emulsifying property of blue lupin protein was determined, and the emulsifying activity index (EAI) was calculated to be 41.78 m²/g, and the emulsifying stability index (ESI) was 15.34 min (Table 1). The EAI value of *L. angustifolius* is reported as 29.3 m²/g and ESI as 14.9 min by Lara-Rivera et al. (2017). It is reported in the literature that, for casein, EAI = 175.6 m²/g and ESI = 33.79 min (Luo, Pan, &

Zhong, 2014). The emulsifying properties of blue lupin protein reported in this paper are lower than that of casein. Can Karaca, Low, and Nickerson (2011) studied the emulsifying properties of proteins from different legumes (chickpea, faba bean, lentil and pea) that are similar to the results reported in this paper. The salt extracted protein samples showed lower EAI values (33.8–43 m²/g) and ESI values (11–25 min) for most legumes. Emulsifying properties depend on protein content, surface charge, solubility and hydrophobicity. Lampart-Szczapa et al. (2006) observed emulsifying activities of three lupin species in the range of 11.4–12.2 m²/g and EAI is 7.4–8.4 min. *L. angustifolius* showed better emulsifying activity than white lupin varieties indicating its higher surface hydrophobicity (Lampart-Szczapa et al., 2006; Lara-Rivera et al., 2017).

Food emulsions are used in many foods such as butter, mayonnaise, cheese, salad dressings, ice cream and in some infant formulas. Protein molecules form emulsion by reducing the interfacial tension between two liquid phases that leads to the formation of a protective layer around the globules (Smith, 2010; Yi-Shen, Shuai, & FitzGerald, 2018).

3.1.5 | Gelling property

The gelling property of blue lupin protein was measured, and the least gelation capacity was found with 8% protein solution (Table 1). The capacity of food proteins to form gels is generally assessed by studying 'least gelling concentration'. The gelling property of a protein can be varied by change in temperature, pH, ionic strength, nature and concentration of protein and interaction with other molecules. Similar gelling results are reported in the literature for various lupin species, soybean, chickpea and other legumes (Boye et al., 2010; Rodriguez-Ambriz, Martinez-Ayala, Millan, & Davila-Ortiz, 2005; Sathe, Deshpande, & Salunkhe, 1982). The food protein gels are formed by interaction of polymeric molecules and consist of three-dimensional networks that exhibit elasticity and provide textural strength to gels (Damodaran, 1996). Differential scanning calorimetry (DSC) analysis performed to compare the gelling properties of lupin and soy protein isolates indicated that the soy protein is superior in this aspect (Berghout, Boom, & van der Goot, 2015). It is concluded that even though the lupin protein isolate forms weaker gelling networks, it is very appropriate for high protein foods that require low viscosity after heating (Berghout, Boom, & van der Goot, 2015).

These results indicate that blue lupin protein isolate has great potential as a value-added ingredient in food industry.

3.2 | Biological activities of blue lupin protein hydrolysates

As described in Section 2, blue lupin protein was subjected to enzymatic hydrolysis using three enzymes, namely, pepsin, pancreatin and flavourzyme. Hydrolysates collected at hourly intervals (1 to 4 h) were subjected to separation by MWCO

membranes. A total of 48 MWCO fractions were isolated, and each of these fractions were named based on the enzyme used, hydrolysis time and the molecular weight range. For instance, "Pep.5k.1h" indicates the fraction obtained by 1-h pepsin hydrolysis and has a molecular weight range of 3–5 kDa (Table 4). Enzymes chosen for this study were based on their specificity and their significance in human digestive system. A brief description of their activity is provided below.

Pepsin is a principal enzyme of the stomach and is active in acidic environment in the pH range of 1.3 to 2.0. Pepsin's cleavage is more specific at pH 1.3 and its specificity is lost above pH 2. As one of the principal digestive enzymes, pepsin preferentially cleaves proteins at Phe, Tyr, Trp and Leu in position P1 or P1'. Pancreatin is a commercial name for a mixture of digestive enzymes produced in the pancreas. The main principal proteases in pancreatin are trypsin and chymotrypsin. Trypsin is an enzyme with narrow specificity and preferentially cleaves at Arg and Lys in position P1 with higher rates for Arg, especially at high pH. The presence of Pro usually blocks the action when found in position P1' but not when Lys is in position P1 and Trp is in position P2 at the same time (Expasy, 2017). Chymotrypsin is an enzyme with broader specificities. It preferentially cleaves proteins at Trp, Tyr and Phe in position P1 (high specificity) and to a lesser extent at Leu, Met and His in position P1 (Expasy, 2017). Flavourzyme is an endopeptidase and exopeptidase with a broad specificity. The enzyme is commercially sold as a preparation obtained from mold *Aspergillus oryzae* and consists of a mixture of seven peptidases (Merz et al., 2015). As a mixture of peptidases, it is anticipated that flavourzyme will show high hydrolysis rate and cleaves the substrates rapidly at the first stage of hydrolysis when the numbers of peptide bonds are readily available.

TABLE 4 Antimicrobial activities (IC₅₀) (µg/ml) of some active MWCO fractions*

Microorganism	MWCO fractions	IC ₅₀ (µg/ml)
<i>Bacillus cereus</i>	Pan.5k.3h	220 ± 13.20
	Pan.2k.3h	225 ± 9.25
	Pan.2k.4h	198 ± 11.20
	Fl.10k.2h	160 ± 12.05
<i>Staphylococcus aureus</i>	Pep.5k.1h	95 ± 4.30
	Pep.2k.4h	100 ± 12.30
	Pan.5k.2h	120 ± 8.64
	Pan.10k.4h	205 ± 18.43
	Pan.3k.3h	145 ± 13.10
	Pan.3k.4h	170 ± 11.40
	Fl.2k.3h	62 ± 7.21
	Fl.2k.4h	295 ± 14.80

Note: *All values are mean of triplicate determination ± standard deviation ($p \leq 0.05$).

Abbreviation: MWCO, molecular weight cut-off; Numbers in bold represent significantly lower IC₅₀ values.

3.2.1 | ACE inhibitory activities

The enzymatic hydrolysis of blue lupin protein followed by MWCO fractionation produced 48 fractions. ACE inhibitory properties of these MWCO fractions have been determined and the results are presented in Figures 1–3 and Table 2.

The IC_{50} values of MWCO fractions obtained from lupin protein hydrolysates ranged from 450 ± 11 to 1400 ± 21 $\mu\text{g/ml}$ (Table 2). The IC_{50} values of MWCO fractions obtained from pepsin hydrolysates ranged from 520 ± 25 to 1103 ± 29 $\mu\text{g/ml}$ (Table 2). The lowest IC_{50} value was obtained from 3 to 5 kDa fraction of 2-h hydrolysis (520 ± 25 $\mu\text{g/ml}$) (highest activity). The IC_{50} values obtained from pancreatin hydrolysis ranged from 450 ± 11 to 1400 ± 21 $\mu\text{g/ml}$ (Table 2). The lowest IC_{50} value was obtained from 2 to 3 kDa fraction of 4-h hydrolysis (450 ± 11 $\mu\text{g/ml}$). The IC_{50} values obtained from flavourzyme hydrolysis ranged from 600 ± 18 to 1210 ± 27 $\mu\text{g/ml}$ (Table 2). The lowest IC_{50} value was obtained from 3 to 5 kDa fraction of 1-h hydrolysis (600 ± 18 $\mu\text{g/ml}$).

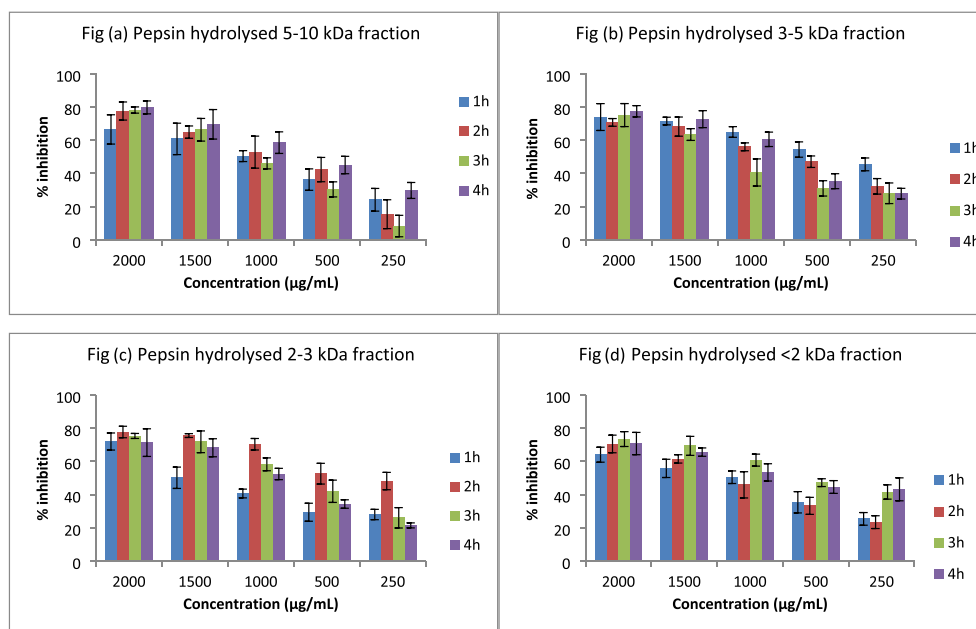
Results from ACE inhibitory screening of blue lupin protein hydrolysates showed that all fractions exhibit ACE inhibitory activities of different strengths. In general, low IC_{50} values (best activities) were observed in most 4-h hydrolysates regardless of their molecular weight ranges. This trend was expected as prolonged hydrolysis will result in the increased numbers of peptides produced that are likely to contribute to the strength of their ACE inhibitory properties. The presence of numerous peptides with different sequences may also cumulatively affect the ACE inhibitory activity values of various MWCO fractions. These results are in agreement with the results reported in the literature on different protein sources (Kamran, Salampessy, & Reddy, 2016).

The ACE inhibitory activity of lupin protein hydrolysates varied widely and was significantly different ($p < 0.05$) by the enzymes used,

hydrolysis times and MWCO fractions. The 2–3 kDa fraction of 4-h pancreatin hydrolysis exhibited the lowest IC_{50} value (450 ± 11 $\mu\text{g/ml}$). As discussed before, pancreatin is a mixture of the three main digestive enzymes: pepsin, trypsin and chymotrypsin. It is, therefore, expected that this combination of the three enzymes will produce smaller peptides due to the cumulative specificities these enzymes possess. Herrera Chalé, Ruiz Ruiz, Acevedo Fernández, Betancur Ancona, and Segura Campos (2014) have reported the use of pepsin–pancreatin mixture to hydrolyse *Mucuna pruriens* proteins. Their ACE inhibitory activities were significant with the best IC_{50} value of 19.5 $\mu\text{g/ml}$. In other research with peanut proteins, Quist, Phillips, and Saalia (2009) reported highly significant ACE inhibitory activities with IC_{50} values of 7.9–65.9 $\mu\text{g/mL}$, and 11–36 $\mu\text{g/mL}$ for raw and roasted peanut, respectively, with pepsin–pancreatin hydrolysis. These reported findings are better than the results from the present study on the ACE inhibitory activities of blue lupin-derived peptides with pancreatin.

In general, pepsin produced a number of active peptide fractions (Table 2). Pepsin is a digestive protease present in animals and humans and is the main proteolytic enzyme in the stomach. Pepsin exhibits broad specificity and can produce peptides with different amino acid residues at both C- and N-termini. Consequently, peptides with different affinities towards ACE are expected to be present in pepsin protein hydrolysates. Boschin, Scigliuolo, Resta, and Arnoldi (2014a) reported the ACE inhibitory activities of some industrial lupin protein isolates and purified protein fractions were significant with IC_{50} values as low as 138 $\mu\text{g/ml}$. Chiang, Tsou, Tsai, and Tsai (2006) had also reported the use of pepsin to hydrolyse soybean proteins and demonstrated that pepsin is capable of cleaving legume proteins to produce bioactive peptides with ACE inhibitory properties and support the present findings on the peptides produced from blue lupin protein by this enzyme.

FIGURE 1 Angiotensin converting enzyme (ACE) inhibition (%) of lupin protein fractions hydrolysed by pepsin ($n = 3$; $p \leq 0.05$). (Standard deviation is represented as error bars on each data point)



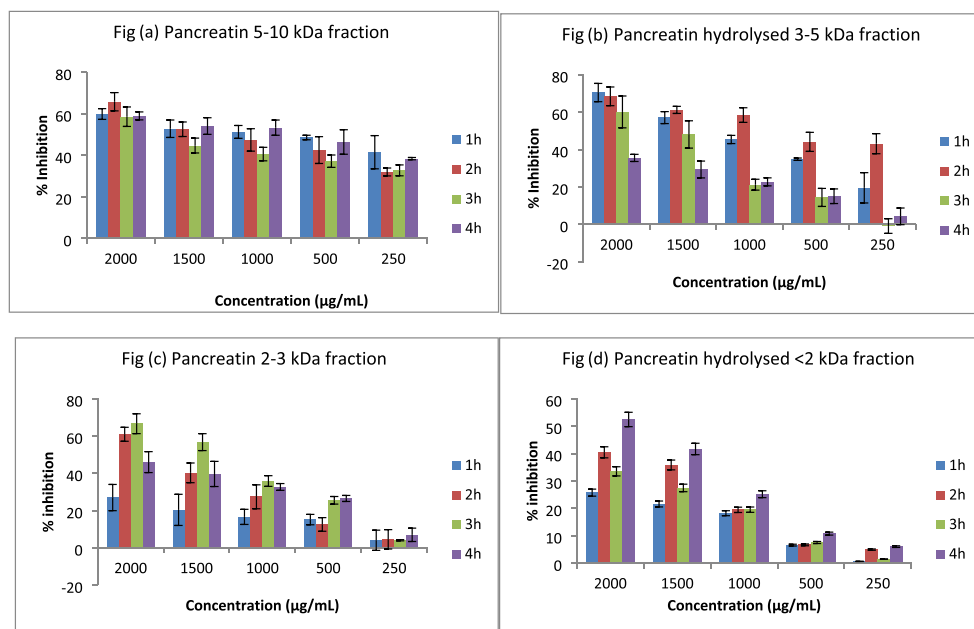


FIGURE 2 Angiotensin converting enzyme (ACE) inhibition (%) of lupin protein fractions hydrolysed by pancreatin ($n = 3$; $p \leq 0.05$). (Standard deviation is represented as error bars on each data point)

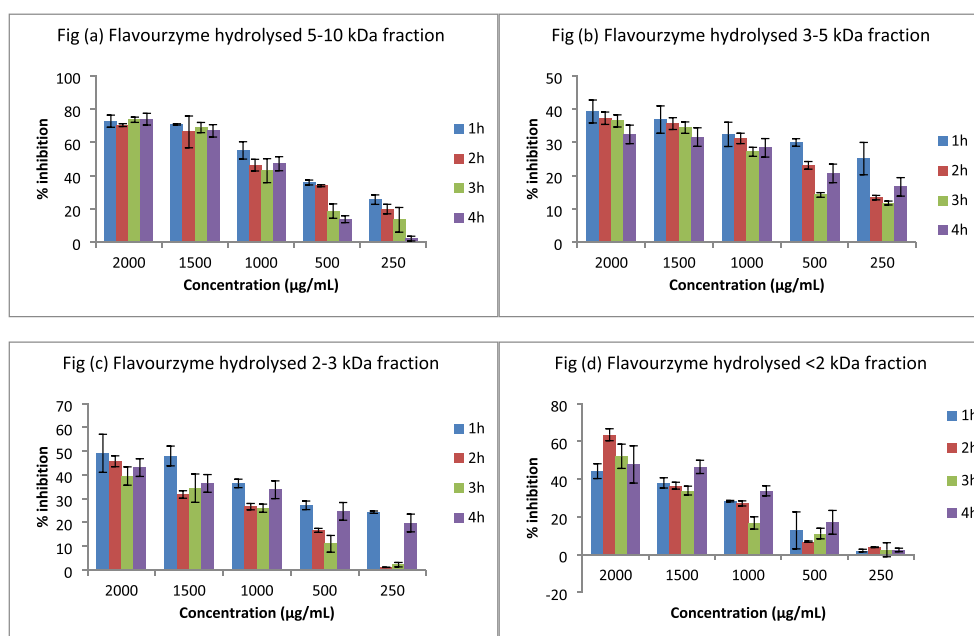


FIGURE 3 Angiotensin converting enzyme (ACE) inhibition (%) of lupin protein fractions hydrolysed by flavourzyme ($n = 3$; $p \leq 0.05$). (Standard deviation is represented as error bars on each data point)

Due to its broad specificity, flavourzyme was expected to produce peptides with different affinities towards ACE. Flavourzyme has been shown to produce ACEIPs with lower IC_{50} values due to its broad specificity and hence may cleave the active peptides from either C- or N-terminal ends (Chiang, Tsou, Tsai, & Tsai, 2006). Suh, Whang, Kim, Bae, and Noh (2003) have indeed reported an increase in ACE inhibitory activity of corn gluten protein hydrolysed by flavourzyme. In this study, flavourzyme hydrolysates of blue lupin proteins showed lower ACE inhibitory activity similar to soybean proteins (Chiang, Tsou, Tsai, & Tsai, 2006). These results suggest that soybean and lupin proteins share some similarities in their amino acid sequences as these two plants belong to the same *Fabaceae* family.

3.2.2 | Antimicrobial activity

Antimicrobial activity screening was carried out on the MWCO fractions against *E. coli*, *B. cereus*, *S. aureus* and *C. albicans*, and the results (% inhibition at 1000 $\mu\text{g/mL}$) are presented in Table 3. It was observed from the results of antimicrobial screening that the lupin peptide fractions have better inhibitory activity towards Gram-positive bacteria (*B. cereus* and *S. aureus*) as compared with Gram-negative bacteria (*E. coli*). Eight fractions showed >85% inhibition against *S. aureus*, of which four are pancreatin hydrolysed fractions (4 h: 5–10 kDa, 2 h: 3–5 kDa, 3 h: 2–3 kDa and 4 h: 2–3 kDa), two are from pepsin hydrolysates (1 h: 3–5 kDa and 4 h: <2 kDa) and two are flavourzyme

TABLE 2 Angiotensin converting enzyme (ACE) inhibitory activities (IC_{50}) of lupin protein hydrolysates

Fractions	IC_{50} ($\mu\text{g/ml}$) ($n = 3$) ($p \leq 0.05$)*		
	Pepsin hydrolysates	Pancreatin hydrolysates	Flavourzyme hydrolysates
1 h: 5–10 kDa	1103 \pm 29	800 \pm 27	740 \pm 12
2 h: 5–10 kDa	950 \pm 39	1000 \pm 32	750 \pm 20
3 h: 5–10 kDa	720 \pm 19	1400 \pm 21	1050 \pm 23
4 h: 5–10 kDa	707 \pm 23	700 \pm 18	780 \pm 17
1 h: 3–5 kDa	640 \pm 19	1000 \pm 20	600 \pm 18
2 h: 3–5 kDa	520 \pm 25	950 \pm 17	650 \pm 16
3 h: 3–5 kDa	600 \pm 14	1050 \pm 23	670 \pm 13
4 h: 3–5 kDa	550 \pm 10	550 \pm 10	800 \pm 15
1 h: 2–3 kDa	1035 \pm 21	700 \pm 15	1000 \pm 22
2 h: 2–3 kDa	600 \pm 10	1150 \pm 29	720 \pm 19
3 h: 2–3 kDa	600 \pm 17	1000 \pm 13	700 \pm 14
4 h: 2–3 kDa	560 \pm 14	450 \pm 11	800 \pm 18
1 h: <2 kDa	760 \pm 27	700 \pm 20	950 \pm 20
2 h: <2 kDa	900 \pm 28	1100 \pm 29	1210 \pm 27
3 h: <2 kDa	700 \pm 9	1000 \pm 14	1200 \pm 17
4 h: <2 kDa	660 \pm 14	1110 \pm 16	650 \pm 11

Note: *All values are mean of triplicate determination \pm standard deviation.

hydrolysed fractions (3 h: <2 kDa and 4 h: <2 kDa) that are the promising candidates (Table 3). In case of *B. cereus* inhibition, three pancreatin hydrolysed fractions (3 h: 3–5 kDa, 3 h: <2 kDa and 4 h: <2 kDa) and one flavourzyme (2 h: 5–10 kDa) fraction showed >80% inhibition against Gram-positive bacteria, *B. cereus* (Table 3). Pepsin hydrolysed fractions showed lower *B. cereus* inhibition as compared with pancreatin and flavourzyme hydrolysed fractions. The most active antimicrobial pepsin hydrolysed fraction showed $63.1 \pm 0.02\%$ inhibition that was produced after 2 h hydrolysis with 2–3 kDa molecular weight range. The results of Gram-negative bacterial inhibition showed that pepsin hydrolysed fractions (3 h: 3–5 kDa and 4 h: 3–5 kDa) displayed 55.9 ± 0.04 and 65.9 ± 0.03 , respectively, against *E. coli*. Overall, pepsin hydrolysed MWCO fractions are the only ones that were active against Gram-negative bacterial growth. All the three enzymes showed activities against Gram-positive bacterial growth with pancreatin producing largest number of active fractions for this class of bacteria.

No significant activity was observed against *C. albicans* (no antifungal activity) by any of the 48 MWCO fractions tested. The maximum observed % inhibition activity was $11.4 \pm 0.04\%$, with most active fraction being pancreatin hydrolysed for 4 h: 5–10 kDa. Overall, the results presented in this research are comparable with other food peptides with significant inhibition against Gram-positive bacteria and minimal inhibition towards the Gram-negative bacteria and *C. albicans* which is a fungus (Salampey, 2010).

Research by McClean, Beggs, and Welch (2014) evaluated four food derived peptides from soybean, barley, α -casein and α -zein for antimicrobial activity against *Micrococcus luteus*, *S. aureus* and *E. coli*, and the yeast, *C. albicans*. The peptides TTMLPW (from α -casein) and PGTA VFK (from soybean) inhibited growth of all four microorganisms

tested, whereas EVSLNSGY (from barley) inhibited the growth of three bacteria but was ineffective against the yeast. The peptide from α -zein (VHLPP) showed no antimicrobial activity (McClean, Beggs, & Welch, 2014). The peptide PGTA VFK from soybean showed the highest antimicrobial activity of all the peptides tested with lowest MIC value ($31 \mu\text{M}$) with all three bacteria and $201 \mu\text{M}$ with *C. albicans*.

The barley protein hydrolysate fraction (10 kDa) was studied by Bamdad, Sun, Guan, and Chen (2015) for its antimicrobial activity against nonbeneficial bacteria (*E. coli* and *M. luteus*) and beneficial lactic acid bacteria (*Lactobacillus reuteri* and *Lactococcus lactis*). The cationized modified peptides exhibited significantly reduced MIC compared with the unmodified original peptides against *E. coli* and *M. luteus*, indicating they can effectively prevent the growth of nonbeneficial bacteria. The modified peptides had significantly higher MICs (lower inhibition) towards beneficial bacteria compared with those of nonbeneficial bacteria (Bamdad, Sun, Guan, & Chen, 2015).

In this study, the lupin protein hydrolysates that showed $\geq 85\%$ inhibition (Table 3) were further studied for evaluating their IC_{50} values. The least IC_{50} value was obtained by Fl.2k.3h fraction ($62 \pm 7.21 \mu\text{g/ml}$) followed by Pep.5k.1h fraction ($95 \pm 4.30 \mu\text{g/ml}$) against *S. aureus* (Table 4). Other fractions showed IC_{50} values in the range of 100 ± 12.30 to $295 \pm 14.80 \mu\text{g/ml}$ against *S. aureus*. The IC_{50} values of fractions estimated against *B. cereus* are 160 ± 12.05 to $225 \pm 9.25 \mu\text{g/ml}$ (Table 4).

4 | CONCLUSION

The results on functional properties of lupin seed protein and biological properties of protein hydrolysates indicate their tremendous

TABLE 3 Inhibition (%) of microbial growth by lupin protein hydrolysates MWCO fractions (n = 5) (p ≤ 0.05)*

Bacterial cultures	Enzymes		Pepsin hydrolysates				Pancreatin hydrolysates				Flavourzyme hydrolysates			
	Time (h)													
	1	2	3	4	1	2	3	4	1	2	3	4		
MWCO fractions (kDa)														
Escherichia coli	5-10	-3.7 ± 0.04	42.7 ± 0.05	26.6 ± 0.02	53.6 ± 0.04	1.7 ± 0.02	-1.4 ± 0.02	1.9 ± 0.002	18.1 ± 0.03	-5.1 ± 0.002	19.0 ± 0.02	24.3 ± 0.02	23.7 ± 0.32	
	3-5	23.4 ± 0.01	27.6 ± 0.04	55.9 ± 0.04	65.9 ± 0.03	16.3 ± 0.04	20.5 ± 0.03	27.1 ± 0.02	38.5 ± 0.05	49.2 ± 0.04	50.3 ± 0.04	18.4 ± 0.01	22.7 ± 0.21	
	2-3	46.0 ± 0.2	29.5 ± 0.11	48.2 ± 0.03	-3.8 ± 0.02	22.7 ± 0.03	26.2 ± 0.02	15.5 ± 0.05	5.6 ± 0.05	29.1 ± 0.03	17.0 ± 0.03	14.9 ± 0.04	27.6 ± 0.34	
	<2	45.3 ± 0.3	-4.0 ± 0.01	24.6 ± 0.02	59.8 ± 0.02	48.1 ± 0.09	33.9 ± 0.04	27.0 ± 0.06	24.0 ± 0.04	20.4 ± 0.01	22.9 ± 0.04	-1.7 ± 0.05	53.6 ± 0.22	
Bacillus cereus	5-10	8.2 ± 0.02	9.5 ± 0.02	51.0 ± 0.03	53.0 ± 0.02	15.6 ± 0.07	27.0 ± 0.02	24.8 ± 0.04	48.7 ± 0.04	17.5 ± 0.03	89.5 ± 0.01	14.7 ± 0.04	31.9 ± 0.02	
	3-5	9.4 ± 0.03	58.9 ± 0.04	40.7 ± 0.02	6.5 ± 0.03	28.2 ± 0.03	3.3 ± 0.04	87.2 ± 0.34	31.2 ± 0.03	23.0 ± 0.04	7.5 ± 0.011	6.9 ± 0.03	29.9 ± 0.03	
	2-3	39.0 ± 0.11	63.1 ± 0.02	50.6 ± 0.03	4.8 ± 0.05	13.7 ± 0.02	13.1 ± 0.02	14.0 ± 0.03	0.9 ± 0.002	26.3 ± 0.05	12.7 ± 0.01	21.0 ± 0.02	6.5 ± 0.04	
	<2	34.7 ± 0.3	32.7 ± 0.05	43.0 ± 0.03	37.9 ± 0.01	53.3 ± 0.01	22.9 ± 0.07	84.1 ± 0.22	85.0 ± 0.05	37.4 ± 0.03	46.9 ± 0.02	5.9 ± 0.03	50.1 ± 0.02	
Staphylococcus aureus	5-10	74.8 ± 0.07	80.1 ± 0.04	81.6 ± 0.02	13.4 ± 0.007	2.0 ± 0.04	71.8 ± 0.03	83.0 ± 0.63	87.7 ± 0.07	22.2 ± 0.02	10.3 ± 0.02	27.4 ± 0.04	23.9 ± 0.02	
	3-5	85.1 ± 0.21	4.0 ± 0.002	3.4 ± 0.11	7.5 ± 0.02	16.8 ± 0.03	86.0 ± 0.67	24.1 ± 0.04	74.7 ± 0.45	5.8 ± 0.011	17.9 ± 0.06	23.1 ± 0.03	14.2 ± 0.03	
	2-3	-0.4 ± 0.02	2.7 ± 0.003	-1.2 ± 0.03	-4.7 ± 0.01	2.3 ± 0.05	60.8 ± 0.05	88.8 ± 0.42	87.6 ± 0.32	36.4 ± 0.02	72.9 ± 0.42	82.7 ± 0.02	13.8 ± 0.02	
	<2	63.5 ± 0.08	15.9 ± 0.02	20.7 ± 0.04	85.7 ± 0.08	82.6 ± 0.21	83.3 ± 0.08	44.1 ± 0.06	19.0 ± 0.03	7.0 ± 0.03	33.0 ± 0.02	87.7 ± 0.01	88.8 ± 0.23	

Note: *All values are mean of triplicate determination ± standard deviation. Numbers in bold represent excellent inhibition. Abbreviation: MWCO, molecular weight cut-off.

potential for the preparation of functional foods and nutraceutical formulations.

Pancreatin hydrolysed fractions displayed best antimicrobial activities with seven fractions exhibiting excellent inhibition against *B. cereus* and *S. aureus*. Pepsin hydrolysed fractions were found to be more active against Gram-negative bacterial growth, and pancreatin and flavourzyme derived fractions were better for their Gram-positive bacterial inhibition. To the best of our knowledge, this is the first study on antimicrobial activities of lupin seed protein hydrolysates.

Overall, the results on ACE inhibitory and antimicrobial activities highlight the potential of incorporating lupin seed protein and protein hydrolysates into food products as preventative agents towards hypertension and microbial diseases. It is concluded that, lupin is an affordable and competitive ingredient for the preparation of nutraceutical and functional foods.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest with respect to the research, authorship and/or publication of this article.

AUTHOR CONTRIBUTIONS

Planning research: Fozia Kamran, Narsimha Reddy and Michael Phillips. Experimental design: Fozia Kamran and Narsimha Reddy. Experimental design for antimicrobial assays: Michael Phillips. Conducting laboratory work: Fozia Kamran. Analysis of results: Fozia Kamran and Narsimha Reddy. Draft manuscript preparation and final corrections: Fozia Kamran. Editing the manuscript: Narsimha Reddy and Michael Phillips.

ETHICS STATEMENT

This research did not involve any human or animal ethics issues to be considered.

DATA AVAILABILITY STATEMENT

Complete raw data and the processed data related to this publication are available with authors.

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REFERENCES

- Andrews, J. M. (2001). Determination of minimum inhibitory concentrations. *Journal of Antimicrobial Chemotherapy*, 48(suppl 1), 5–16. https://doi.org/10.1093/jac/48.suppl_1.5
- Arnoldi, A., Boschin, G., Zanoni, C., & Lammi, C. (2015). The health benefits of sweet lupin seed flours and isolated proteins. *Journal of Functional Foods*, 18, 550–563. <https://doi.org/10.1016/j.jff.2015.08.012>
- Bader, S., Oviedo, J. P., Pickardt, C., & Eisner, P. (2011). Influence of different organic solvents on the functional and sensory properties of lupin (*Lupinus angustifolius* L.) proteins. *LWT Food Science and Technology*, 44(6), 1396–1404. <https://doi.org/10.1016/j.lwt.2011.01.007>
- Balti, R., Bougateg, A., Sila, A., Guillochon, D., Dhulster, P., & Nedjar-Arroume, N. (2015). Nine novel angiotensin I-converting enzyme (ACE) inhibitory peptides from cuttlefish (*Sepia officinalis*) muscle protein hydrolysates and antihypertensive effect of the potent active peptide in spontaneously hypertensive rats. *Food Chemistry*, 170, 519–525. <https://doi.org/10.1016/j.foodchem.2013.03.091>
- Bamdad, F., Sun, X., Guan, L. L., & Chen, L. (2015). Preparation and characterization of antimicrobial cationized peptides from barley (*Hordeum vulgare* L.) proteins. *LWT - Food Science and Technology*, 63(1), 29–36. <http://doi.org/10.1016/j.lwt.2015.03.012>
- Barbana, C., & Boye, J. I. (2011). Angiotensin I-converting enzyme inhibitory properties of lentil protein hydrolysates: Determination of the kinetics of inhibition. *Food Chemistry*, 127(1), 94–101. <https://doi.org/10.1016/j.foodchem.2010.12.093>
- Berghout, J. A. M., Boom, R. M., & van der Goot, A. J. (2015). Understanding the differences in gelling properties between lupin protein isolate and soy protein isolate. *Food Hydrocolloids*, 43(0), 465–472. <https://doi.org/10.1016/j.foodhyd.2014.07.003>
- Boschin, G., Scigliuolo, G. M., Resta, D., & Arnoldi, A. (2014a). ACE-inhibitory activity of enzymatic protein hydrolysates from lupin and other legumes. *Food Chemistry*, 145(0), 34–40. <https://doi.org/10.1016/j.foodchem.2013.07.076>
- Boschin, G., Scigliuolo, G. M., Resta, D., & Arnoldi, A. (2014b). Optimization of the enzymatic hydrolysis of lupin (*Lupinus*) proteins for producing ACE-inhibitory peptides. *Journal of Agricultural and Food Chemistry*, 62(8), 1846–1851. <https://doi.org/10.1021/jf4039056>
- Boye, J. I., Aksay, S., Roufik, S., Ribereau, S., Mondor, M., Farnworth, E., & Rajamohamed, S. H. (2010). Comparison of the functional properties of pea, chickpea and lentil protein concentrates processed using ultrafiltration and isoelectric precipitation techniques. *Food Research International*, 43(2), 537–546. <https://doi.org/10.1016/j.foodres.2009.07.021>
- Can Karaca, A., Low, N., & Nickerson, M. (2011). Emulsifying properties of chickpea, faba bean, lentil and pea proteins produced by isoelectric precipitation and salt extraction. *Food Research International*, 44(9), 2742–2750. <https://doi.org/10.1016/j.foodres.2011.06.012>
- Chao, D., Jung, S., & Aluko, R. E. (2018). Physicochemical and functional properties of high pressure-treated isolated pea protein. *Innovative Food Science & Emerging Technologies*, 45, 179–185. <https://doi.org/10.1016/j.ifset.2017.10.014>
- Chiang, W.-D., Tsou, M.-J., Tsai, Z.-Y., & Tsai, T.-C. (2006). Angiotensin I-converting enzyme inhibitor derived from soy protein hydrolysate and produced by using membrane reactor. *Food Chemistry*, 98(4), 725–732. <https://doi.org/10.1016/j.foodchem.2005.06.038>
- Chin, Y. Y., Chew, L. Y., Toh, G. T., Salampessy, J., Azlan, A., & Ismail, A. (2019). Nutritional composition and angiotensin converting enzyme inhibitory activity of blue lupin (*Lupinus angustifolius*). *Food Bioscience*, 31, 100401. <https://doi.org/10.1016/j.fbio.2019.04.002>
- Damodaran, S. (1996). Functional properties. In S. Nakai, & H. W. Modler (Eds.), *Food proteins: Properties and characterization* (pp. 167–234). Vch Pub.
- El-Adawy, T. A., Rahma, E. H., El-Bedawey, A. A., & Gafar, A. F. (2001). Nutritional potential and functional properties of sweet and bitter lupin seed protein isolates. *Food Chemistry*, 74(4), 455–462. [https://doi.org/10.1016/S0308-8146\(01\)00163-7](https://doi.org/10.1016/S0308-8146(01)00163-7)
- Expasy. 2017, PeptideCutter, http://web.expasy.org/peptide_cutter/
- Gibbs, B. F., Zougman, A., Masse, R., & Mulligan, C. (2004). Production and characterization of bioactive peptides from soy hydrolysate and soy-fermented food. *Food Research International*, 37(2), 123–131.
- Hancock, R. E. W., & Chapple, D. S. (1999). Peptide antibiotics. *Antimicrobial Agents and Chemotherapy*, 43(6), 1317–1323. <https://doi.org/10.1128/AAC.43.6.1317>
- Herrera Chalé, F. G., Ruiz Ruiz, J. C., Acevedo Fernández, J. J., Betancur Ancona, D. A., & Segura Campos, M. R. (2014). ACE inhibitory, hypotensive and antioxidant peptide fractions from *Mucuna pruriens* proteins. *Process Biochemistry*, 49(10), 1691–1698. <https://doi.org/10.1016/j.procbio.2014.06.021>
- Hong, F., Ming, L., Yi, S., Zhanxia, L., Yongquan, W., & Chi, L. (2008). The antihypertensive effect of peptides: A novel alternative to drugs?

- Peptides, 29(6), 1062–1071. <https://doi.org/10.1016/j.peptides.2008.02.005>
- Jayasena, V., Leung, P., & Nasar-Abbas, S. (2010). Effect of lupin flour substitution on the quality and sensory acceptability of instant noodles. *Journal of Food Quality*, 33(6), 709–727. <https://doi.org/10.1111/j.1745-4557.2010.00353.x>
- Jayasena, V., & Nasar-Abbas, S. (2011). Effect of lupin flour incorporation on the physical characteristics of dough and biscuits. *Quality Assurance and Safety of Crops & Foods*, 3(3), 140–147. <https://doi.org/10.1111/j.1757-837X.2011.00100.x>
- Johnson, S. K., McQuillan, P. L., Sin, J. H., & Ball, M. J. (2003). Sensory acceptability of white bread with added Australian sweet lupin (*Lupinus angustifolius*) kernel fibre and its glycaemic and insulinaemic responses when eaten as a breakfast. *Journal of the Science of Food and Agriculture*, 83(13), 1366–1372. <https://doi.org/10.1002/jsfa.1552>
- Kamran, F. (2017). Enzymatic hydrolysis of lupin (*Lupinus angustifolius*) protein: Isolation and characterization of bioactive peptides. (PhD Original research thesis). Available in digital format in the Library of Western Sydney University.
- Kamran, F., & Reddy, N. (2018). Bioactive peptides from legumes: Functional and nutraceutical potential. *Recent Advances in Food Science*, 1(3), 134–149.
- Kamran, F., Salampessy, J., & Reddy, N. (2016). Application of NMR spectroscopy for structural characterization of bioactive peptides derived from food protein. In A. Rahman, & M. I. Choudhary (Eds.), *Applications of NMR spectroscopy* (Vol. 5) (pp. 3–76). Bentham Science Publishers Ltd. <https://doi.org/10.2174/9781681082875116050003>
- King, J., Aguirre, C., & De Pablo, S. (1985). Functional properties of lupin protein isolates (*Lupinus albus* cv Multolupa). *Journal of Food Science*, 50(1), 82–87. <https://doi.org/10.1111/j.1365-2621.1985.tb13282.x>
- Korhonen, H., & Pihlanto, A. (2006). Bioactive peptides: Production and functionality. *International Dairy Journal*, 16(9), 945–960. <https://doi.org/10.1016/j.idairyj.2005.10.012>
- Kuba, M., Tana, C., Tawata, S., & Yasuda, M. (2005). Production of angiotensin I-converting enzyme inhibitory peptides from soybean protein with *Monascus purpureus* acid proteinase. *Process Biochemistry*, 40(6), 2191–2196. <https://doi.org/10.1016/j.procbio.2004.08.010>
- Kuba, M., Tanaka, K., Tawata, S., Takeda, Y., & Yasuda, M. (2003). Angiotensin I-converting enzyme inhibitory peptides isolated from tofuyo fermented soybean food. *Bioscience, Biotechnology, and Biochemistry*, 67(6), 1278–1283. <https://doi.org/10.1271/bbb.67.1278>
- Lampart-Szczapa, E., Konieczny, P., Nogala-Kalucka, M., Walczak, S., Kossowska, I., & Malinowska, M. (2006). Some functional properties of lupin proteins modified by lactic fermentation and extrusion. *Food Chemistry*, 96(2), 290–296. <https://doi.org/10.1016/j.foodchem.2005.02.031>
- Lara-Rivera, A. H., Garcia-Alamilla, P., Lagunes-Galvez, L. M., Rodríguez Macías, R., García Lopez, P. M., & Zamora Natera, J. F. (2017). Functional properties of *Lupinus angustifolius* seed protein isolates. *Journal of Food Quality*, 2017, 8–8. <https://doi.org/10.1155/2017/8675814>
- Lavoie, J. L., & Sigmund, C. D. (2003). Minireview: Overview of the renin-angiotensin system—An endocrine and paracrine system. *Endocrinology*, 144(6), 2179–2183. <https://doi.org/10.1210/en.2003-0150>
- Lee, Y. P., Mori, T. A., Puddey, I. B., Sipsas, S., Ackland, T. R., Beilin, L. J. a., & Hodgson, J. M. (2009). Effects of lupin kernel flour-enriched bread on blood pressure: A controlled intervention study. *The American Journal of Clinical Nutrition*, 89, 766–772. <https://doi.org/10.3945/ajcn.2008.26708>
- Luna-Vital, D. A., Mojica, L., González de Mejía, E., Mendoza, S., & Loarca-Piña, G. (2015). Biological potential of protein hydrolysates and peptides from common bean (*Phaseolus vulgaris* L.): A review. *Food Research International*, 76(Part 1), 39–50. <https://doi.org/10.1016/j.foodres.2014.11.024>
- Luo, Y., Pan, K., & Zhong, Q. (2014). Physical, chemical and biochemical properties of casein hydrolyzed by three proteases: Partial characterizations. *Food Chemistry*, 155, 146–154.
- Mäkinen, S., Streng, T., Larsen, L. B., Laine, A., & Pihlanto, A. (2016). Angiotensin I-converting enzyme inhibitory and antihypertensive properties of potato and rapeseed protein-derived peptides. *Journal of Functional Foods*, 25, 160–173. <https://doi.org/10.1016/j.jff.2016.05.016>
- McClean, S., Beggs, L. B., & Welch, R. W. (2014). Antimicrobial activity of antihypertensive food-derived peptides and selected alanine analogues. *Food Chemistry*, 146, 443–447. <http://doi.org/10.1016/j.foodchem.2013.09.094>
- Meira, S. M. M., Daroit, D. J., Helfer, V. E., Corrêa, A. P. F., Segalin, J., Carro, S., & Brandelli, A. (2012). Bioactive peptides in water-soluble extracts of ovine cheeses from Southern Brazil and Uruguay. *Food Research International*, 48(1), 322–329. <https://doi.org/10.1016/j.foodres.2012.05.009>
- Merz, M., Eisele, T., Berends, P., Appel, D., Rabe, S., Blank, I., ... Fischer, L. (2015). Flavourzyme, an enzyme preparation with industrial relevance: Automated nine-step purification and partial characterization of eight enzymes. *Journal of Agricultural and Food Chemistry*, 63(23), 5682–5693. <https://doi.org/10.1021/acs.jafc.5b01665>
- Möller, N., Scholz-Ahrens, K., Roos, N., & Schrezenmeir, J. (2008). Bioactive peptides and proteins from foods: Indication for health effects. *European Journal of Nutrition*, 47(4), 171–182. <https://doi.org/10.1007/s00394-008-0710-2>
- Nakamura, Y., Yamamoto, N., Sakai, K., Okubo, A., Yamazaki, S., & Takano, T. (1995). Purification and characterization of angiotensin I-converting enzyme inhibitors from sour milk. *Journal of Dairy Science*, 78(4), 777–783. [https://doi.org/10.3168/jds.S0022-0302\(95\)76689-9](https://doi.org/10.3168/jds.S0022-0302(95)76689-9)
- Nasri, R., Younes, I., Jridi, M., Trigui, M., Bougatef, A., Nedjar-Arroume, N., ... Karra-Châabouni, M. (2013). ACE inhibitory and antioxidative activities of Goby (*Zosterisessor ophiocephalus*) fish protein hydrolysates: Effect on meat lipid oxidation. *Food Research International*, 54(1), 552–561. <https://doi.org/10.1016/j.foodres.2013.07.001>
- Nongonierma, A. B., & FitzGerald, R. J. (2016). Strategies for the discovery, identification and validation of milk protein-derived bioactive peptides. *Trends in Food Science & Technology*, 50, 26–43. <https://doi.org/10.1016/j.tifs.2016.01.022>
- Paiva, L., Lima, E., Neto, A. I., & Baptista, J. (2016). Isolation and characterization of angiotensin I-converting enzyme (ACE) inhibitory peptides from *Ulva rigida* C. Agardh protein hydrolysate. *Journal of Functional Foods*, 26, 65–76. <https://doi.org/10.1016/j.jff.2016.07.006>
- Pearce, K. N., & Kinsella, J. E. (1978). Emulsifying properties of proteins: Evaluation of a turbidimetric technique. *Journal of Agricultural and Food Chemistry*, 26(3), 716–723. <https://doi.org/10.1021/jf60217a041>
- Pei Gee, C., Casey, A. J., & Johnson, S. K. (2003). Protein quality and physico-functionality of Australian sweet lupin (*Lupinus angustifolius* cv. Gungurru) protein concentrates prepared by isoelectric precipitation or ultrafiltration. *Food Chemistry*, 83(4), 575–583. [https://doi.org/10.1016/S0308-8146\(03\)00156-0](https://doi.org/10.1016/S0308-8146(03)00156-0)
- Pritchard, S. R., Phillips, M., & Kailasapathy, K. (2010). Identification of bioactive peptides in commercial Cheddar cheese. *Food Research International*, 43(5), 1545–1548. <https://doi.org/10.1016/j.foodres.2010.03.007>
- Quist, E. E., Phillips, R. D., & Saalia, F. K. (2009). Angiotensin converting enzyme inhibitory activity of proteolytic digests of peanut (*Arachis hypogaea* L.) flour. *LWT—Food Science and Technology*, 42(3), 694–699. <https://doi.org/10.1016/j.lwt.2008.10.008>
- Rho, S. J., Lee, J. S., Chung, Y. I., Kim, Y. W., & Lee, H. G. (2009). Purification and identification of an angiotensin I-converting enzyme inhibitory peptide from fermented soybean extract. *Process Biochemistry*, 44(4), 490–493. <https://doi.org/10.1016/j.procbio.2008.12.017>
- Rodríguez-Ambríz, S. L., Martínez-Ayala, A. L., Millán, F., & Davila-Ortiz, G. (2005). Composition and functional properties of *Lupinus campestris*

- protein isolates. *Plant Foods for Human Nutrition*, 60(3), 99–107. <https://doi.org/10.1007/s11130-005-6835-z>
- Salampessy, J. (2010). Enzymatic production, purification and analysis of bioactive peptides from fish proteins. (PhD Thesis). Available in digital format in the Library of Western Sydney University.
- Salampessy, J., Phillips, M., Seneweera, S., & Kailasapathy, K. (2010). Release of antimicrobial peptides through bromelain hydrolysis of leatherjacket (*Meuschenia* sp.) insoluble proteins. *Food Chemistry*, 120(2), 556–560. <https://doi.org/10.1016/j.foodchem.2009.10.054>
- Sanjukta, S., & Rai, A. K. (2016). Production of bioactive peptides during soybean fermentation and their potential health benefits. *Trends in Food Science & Technology*, 50, 1–10. <https://doi.org/10.1016/j.tifs.2016.01.010>
- Sathe, S. K., Deshpande, S. S., & Salunkhe, D. K. (1982). Functional properties of lupin seed (*Lupinus mutabilis*) proteins and protein concentrates. *Journal of Food Science*, 47(2), 491–497. <https://doi.org/10.1111/j.1365-2621.1982.tb10110.x>
- Sironi, E., Sessa, F., & Duranti, M. (2005). A simple procedure of lupin seed protein fractionation for selective food applications. *European Food Research and Technology*, 221(1–2), 145–150. <https://doi.org/10.1007/s00217-005-1151-2>
- Shahidi, F., & Zhong, Y. (2008). Bioactive peptides. *Journal of AOAC International*, 91(4), 914–931. <https://doi.org/10.1093/jaoac/91.4.914>
- Smith, D. M. (2010). Protein separation and characterization procedures. In S. S. Nielsen (Ed.), *Food analysis* (pp. 261–281). Springer Science +Business Media, LLC.
- Suh, H. J., Whang, J. H., Kim, Y. S., Bae, S. H., & Noh, D. O. (2003). Preparation of angiotensin I converting enzyme inhibitor from corn gluten. *Process Biochemistry*, 38(8), 1239–1244. [https://doi.org/10.1016/S0032-9592\(02\)00316-3](https://doi.org/10.1016/S0032-9592(02)00316-3)
- Tang, W., Yuan, H., Zhang, H., Wang, L., Qian, H., & Qi, X. (2015). An antimicrobial peptide screened from casein hydrolyzate by *Saccharomyces cerevisiae* cell membrane affinity method. *Food Control*, 50, 413–422. <https://doi.org/10.1016/j.foodcont.2014.09.030>
- Tizazu, H., & Emire, S. A. (2010). Chemical composition, physiochemical and functional properties of lupin (*Lupinus albus*) seeds grown in Ethiopia. *African Journal of Food, Agriculture, Nutrition and Development*, 10(8), 3029–3046.
- Torres, A., Frias, J., Granito, M., Guerra, M., & Vidal-Valverde, C. (2007). Chemical, biological and sensory evaluation of pasta products supplemented with alpha-galactoside-free lupin flours. *Journal of the Science of Food and Agriculture*, 87(1), 74–81. <https://doi.org/10.1002/jsfa.2673>
- Villarino, C. B. J., Jayasena, V., Coorey, R., Chakrabarti-Bell, S., & Johnson, S. (2015). Optimization of formulation and process of Australian sweet lupin (ASL)-wheat bread. *LWT - Food Science and Technology*, 61(2), 359–367. <https://doi.org/10.1016/j.lwt.2014.11.029>
- Wang, S., Lin, J., Ye, M., Ng, T. B., Rao, P., & Ye, X. (2006). Isolation and characterization of a novel mung bean protease inhibitor with anti-pathogenic and anti-proliferative activities. *Peptides*, 27(12), 3129–3136. <https://doi.org/10.1016/j.peptides.2006.07.013>
- Wang, S. Y., Wu, J. H., Ng, T. B., Ye, X. Y., & Rao, P. F. (2004). A non-specific lipid transfer protein with antifungal and antibacterial activities from the mung bean. *Peptides*, 25(8), 1235–1242. <https://doi.org/10.1016/j.peptides.2004.06.004>
- Yeo, I.-C., Lee, N. K., Cha, C.-J., & Hahm, Y. T. (2011). Narrow antagonistic activity of antimicrobial peptide from *Bacillus subtilis* SCK-2 against *Bacillus cereus*. *Journal of Bioscience and Bioengineering*, 112(4), 338–344. <https://doi.org/10.1016/j.jbiosc.2011.06.011>
- Yi-Shen, Z., Shuai, S., & FitzGerald, R. (2018). Mung bean proteins and peptides: Nutritional, functional and bioactive properties. *Food & Nutrition Research*, 62. <https://doi.org/10.29219/fnr.v62.1290>
- Yoshie-Stark, Y., Bez, J., Wada, Y., & Waesche, A. (2004). Functional properties, lipoxigenase activity, and health aspects of *Lupinus albus* protein isolates. *Journal of Agricultural and Food Chemistry*, 52(25), 7681–7689. <https://doi.org/10.1021/jf049583c>

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